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PATENT ABSTRACTS OF JAPAN

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(54) TRANSPLANT BODY FOR REGENERATION OF CARTILAGE TISSUE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a transplant body for regeneration of cartilage tissue which exhibits excellent biocompatibility, is excellent in the seeding properties of cells and clinical handleability and has sufficient mechanical strength.
SOLUTION: This transplant body is constituted by forming a natural high- polymer porous structure in a mesh body consisting of a bioabsorptive synthetic high polymer or an internal structure matrix of a porous body to obtain a carrier for carrying cells, then carrying the cartilage cells or the stem cells differentiating to the cartilage cells to this carrier.

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CLAIMS

[Claim(s)]
[Claim 1] Support for support of the stem cell which specializes in the chondrocyte or chondrocyte which consists of a complex ingredient with which the vesicular structure object which consists of naturally-ocurring polymers further is formed in the internal structure matrix of the mesh object which consists of living body absorptivity synthetic macromolecule, or a porous body.
[Claim 2] Support for support of the stem cell which specializes in the chondrocyte or the cartilaginous tissue according to claim 1 whose complex ingredient is a sheet-like object.
[Claim 3] Support for support of the stem cell to which a complex ingredient specializes in the chondrocyte or the cartilaginous tissue of claim 1 which rolls a sheet-like object according to claim 2 a laminating or in the shape of a roll.
[Claim 4] The transplant for using for cartilage anagenesis which made the support for support of the stem cell which specializes in the chondrocyte or chondrocyte of claim 1-3 given in any 1 term support the stem cell which specializes in chondrocyte or chondrocyte.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the rebirth of the cartilaginous tissue used in order to restore the cartilage wound by causes, such as diseases, such as osteoarthritis, and accident.
[0002]
[Description of the Prior Art] The osteoarthritis is a high ********** disease in the orthopedics field, and often causes an advanced functional disorder. Although an artificial joint operation accomplishes the subject of surgical treatment, an artificial joint part made from a current metal and a current macromolecule polymer has problems, such as infection, wear, slack, and breakage. In the case of implantation, in addition to the problem that a donor is insufficient, when donors are others, there is also a problem of the rejection based on an immune response. By such existence of various troubles, by current, it thinks that the cure by the tissue engineering-technique is ideal, and ** and research on the rebirth of a cartilaginous tissue are done briskly. In order to reproduce a cartilaginous tissue by the tissue engineering-technique, the support ingredient of the three dimension porosity nature as a base material of the body tissue currently formed is required as a scaffold for the stem cell which specializes in chondrocyte or chondrocyte to increase. The three dimension porosity nature support ingredient which conditions, such as porosity nature, biocompatibility, and living body absorptivity, were required, and prepared such a support ingredient conventionally with naturally-ocurring polymers, such as living body absorptivity synthetic macromolecule like the copolymer (PLGA) of polylactic acid (PLA), polyglycolic acid (PGA), a lactic acid, and a glycolic acid or a collagen, is used well.
[0003] However, the thing which consists of the above-mentioned living body absorptivity synthetic macromolecule It is hydrophobicity although excelled in mechanical strength. Moreover, the large clearance and a crack sake, It is very difficult to carry out seeding of the stem cell which most cells pass through a clearance, and do not appear on it, but specializes in chondrocyte or chondrocyte. This sake, Since the rate of seeding of an effective cell was not obtained and these cells were not able to be accumulated on support support in large quantities, the regeneration efficiency of a cartilaginous tissue was low and had become a practically serious failure. On the other hand, although it was a hydrophilic property, and the interaction with a cell was very excellent and seeding of a cell was easy the interaction, since mechanical strength was soft and tended to be twisted low, collagen sponge included the trouble which is the porosity nature ingredient of the naturally-ocurring polymers of the living body origin of being hard to deal with it, by clinical, for example.
[0004]
[Problem(s) to be Solved by the Invention] This invention makes it a technical problem to solve such a trouble of the conventional technique. Seeding of the stem cell which has good biocompatibility and specifically specializes in chondrocyte or chondrocyte is easy. While seeding effectiveness is good, therefore it is possible to accumulate these cells on support support in large quantities and the regeneration efficiency of a cartilaginous tissue is good A mechanical strength is also high and it is in offering the support support of the stem cell which specializes in

the chondrocyte or chondrocyte which is easy to deal with it also in clinical. Furthermore, it is going to offer the living body implantation-graft object for reproducing a cartilaginous tissue which made such an ingredient contain the stem cell which specializes in chondrocyte or chondrocyte.
[0005]
[Means for Solving the Problem] This invention is made in order to solve the above-mentioned technical problem, and it consists of (1) - (4) below.
(1) Support for support of the stem cell which specializes in the chondrocyte or chondrocyte which consists of a complex ingredient with which the vesicular structure object which consists of naturally-ocurring polymers further is formed in the front face of the mesh object which consists of living body absorptivity synthetic macromolecule, or a porous body, and the internal structure matrix.
(2) Support for support of the stem cell whose complex ingredient is a sheet-like object and which specializes in chondrocyte or a cartilaginous tissue given in (1).
(3) Support for support of the stem cell to which a complex ingredient specializes in the chondrocyte or chondrocyte of (1) which winds the sheet-like object of a publication around the above (2) a laminating or in the shape of a roll.
(4) Transplant for using for cartilage anagenesis which made the support for support of the stem cell which specializes in chondrocyte or chondrocyte given [any 1] in (1) - (3) support the stem cell which specializes in chondrocyte or chondrocyte.
[0006] Hereafter, this invention is explained further in full detail. The support which supports the stem cell which specializes in the chondrocyte or chondrocyte in this invention is constituted by the complex ingredient which formed further the vesicular structure object which consists of naturally-ocurring polymers, such as a collagen, in the front face in the mesh object or porous body of living body absorptivity synthetic macromolecule, and its internal structure matrix.
[0007] The mesh object or porous body of living body absorptivity synthetic macromolecule used for this invention is used in order to mainly increase the mechanical strength of the complex ingredient of this invention, and a mesh object is easy to consist of textiles, textile fabrics, or a nonwoven fabric. Moreover, a porous body can be obtained by the well-known approaches, such as the foaming method for using a foaming agent, or a porosity-ized agent removal method. In the foaming casting method of this porous body, after adding a foaming agent to a high molecular compound and making a foaming agent foam to it, the above-mentioned macromolecule is stiffened. What is necessary is to add a water-soluble saccharide or salts and just to carry out washing removal of this water-soluble matter with water after hardening into a polymer solution. Since the pore consistency of the naturally-ocurring-polymers vesicular structure object per mesh unit becomes high and a seeding cell is held the more at this pore although a mechanical strength falls the more the magnitude of the friend eye of a mesh or the magnitude of the hole of a porous body becomes large, the number of seeding cells in complex can be increased, and the rebirth of a cartilaginous tissue becomes efficient. Therefore, the magnitude of the friend eye of the mesh or the magnitude of the hole of a porous body takes into consideration the mechanical strength called for, resiliency, or the reproduction speed of a cartilaginous tissue according to the location in the living body transplanted, and is defined suitably.
[0008] As living body absorptivity synthetic macromolecule which forms a mesh object or a porous body, polysaccharide, such as polyester, such as a copolymer of polylactic acid, polyglycolic acid, a lactic acid, and a glycolic acid, the Pori malic acid, and a Polly epsilon-caprolactone, or a cellulose, and the Pori alginic acid, etc. can be mentioned. The living body absorptivity synthetic macromolecule preferably used in this invention is [0009] which is the copolymer of polylactic acid, polyglycolic acid, a lactic acid, and a glycolic acid. Although all can be used if the naturally-ocurring-polymers vesicular structure object of this invention originates in a living body and biocompatibility is shown, a collagen, gelatin, fibronectin and one or more sorts of things chosen from the laminin, especially a collagen are used preferably. Although there is a thing of I, II, III, and IV mold in a collagen, these all can be used in this invention. Pore of a naturally-ocurring-polymers vesicular structure object is made into growth of a seeding cell, and the scaffold of anagenesis, and, as for pore, continuing is desirable. The magnitude is preferably

good to be referred to [1-300-micrometer] as about 20-100 micrometers. Moreover, in this invention, although what is necessary is just to define thickness suitably by the use mode of living body composite material, it is usually 0.1-1mm preferably 0.1-5mm. The voidage is usually 80% or more.

[0010] The complex ingredient of this invention can be obtained by carrying out the crosslinking bond of the mesh object, or a porous body and a naturally-ocurring-polymers vesicular structure object of said living body absorptivity synthetic macromolecule, for example, although the porous body which consists of naturally-ocurring polymers, such as a collagen, can be further formed in the internal structure matrix of the mesh object of living body absorptivity synthetic macromolecule, or a porous body, i.e., the friend eye of a mesh object, or the hole of a porous body, and the complex ingredient of this invention can be manufactured by various approaches. After this approach adheres and makes the solution of naturally-ocurring-polymers ingredients, such as a collagen, sink into the mesh object or porous body of (1) living-body absorptivity synthetic macromolecule, it carries out (2) freeze drying and, subsequently processes the living body composite material which carries out (3) generation by the gas chemistry cross linking agent. In the above-mentioned process (1), said living body absorptivity synthetic macromolecule mesh object is processed in the naturally-ocurring-polymers water solution of said living body origin. Although there are various things as an art, dip coating and the applying method are adopted preferably. Dip coating is effective when the concentration and viscosity of a naturally-ocurring-polymers water solution of the living body origin are low, and specifically, it is performed by immersing a living body absorptivity synthetic macromolecule mesh object in the low concentration water solution of the naturally-ocurring polymers of the living body origin. Its concentration and viscosity of a naturally-ocurring-polymers water solution of the living body origin are high, the applying method is effective when dip coating is inapplicable, and specifically, it is performed by applying the high concentration water solution of the naturally-ocurring polymers of the living body origin to a living body absorptivity synthetic macromolecule mesh object.

[0011] Subsequently to (2) freeze drying, the composite with which the naturally-ocurring-polymers solution sank in and adhered to the living body absorptivity synthetic macromolecule mesh object or the porous body is attached. Although freeze drying freezes the above-mentioned composite and this is freeze-dried under vacuum reduced pressure, the naturally-ocurring polymers of the living body origin are porosity-ized by this process, and the complex ingredient of the mesh object of living body absorptivity synthetic macromolecule, or a porous body and a naturally-ocurring-polymers vesicular structure object is formed of it.

[0012] A well-known approach can apply the approach of freeze drying as it is conventionally. Freezing temperature is usually -20 degrees C or less. A freeze-drying pressure is usually prepared under reduced pressure of 0.2Torr extent that what is necessary is just to set up the reduced pressure conditions from which the frozen water serves as a gas. Subsequently to the bridge formation process of (3), the freeze-dried complex ingredient is attached. This process is required in order to give the sufficient elasticity and the reinforcement for the vesicular structure of the bridge complex ingredient which heightens bonding strength with a synthetic macromolecule mesh object, and is considered as a request being stabilized, while bridge-formation-izing the naturally-ocurring-polymers porous body of the living body origin which constitutes a compound biomaterial by the gas cross linking agent and hardening the vesicular structure object of naturally-ocurring polymers.

[0013] Generally, although the chemistry cross-linking method using the bridge formation-ized agent and the gas bridge formation-ized agent of the shape of physical cross-linking methods, such as optical bridge formation, heat bridge formation, etc. by UV irradiation processing, and a solution etc. is known as the bridge formation-ized approach, in this invention, the approach using a gas bridge formation-ized agent is the most desirable.

[0014] That is, it is because there is a possibility that naturally-ocurring polymers may dissolve in the bridge formation process, by the approach of a degree of cross linking being restricted to a bridge formation chemically-modified [the] degree, and there being a possibility of causing the deterioration and decomposition of living body absorptivity synthetic macromolecule which

constitute a compound biomaterial further, in physical cross-linking methods, such as optical bridge formation, heat bridge formation, etc. by UV irradiation processing, and using a solution-like bridge formation-ized agent also with a chemistry cross-linking method. In addition, even if it adopted the approach of giving optical bridge formation and heat bridge formation to ** which prevents the dissolution of the bridge formation-ized agent solution of naturally-ocurring polymers in advance of bridge formation by the solution-like bridge formation-ized agent, since decomposition and deterioration of naturally-ocurring polymers arise with light or heat as described above, it is not desirable.

[0015] On the other hand, without conquering all the above troubles and producing decomposition and deterioration, the approach using a gas cross linking agent can obtain the bridge-formation-ized complex ingredient which has sufficient reinforcement which is sufficient for bonding strength with a synthetic macromolecule mesh object being heightened, and considering as the purpose, and elasticity while a bridge is constructed over naturally-ocurring polymers and they are three-dimension-ized with a desired gestalt. As a cross linking agent used by this invention, each well-known thing can use it conventionally. The cross linking agents used preferably are the aldehydes like glutaraldehyde, formaldehyde, and a paraformaldehyde, especially glutaraldehyde.

[0016] Bridge formation-ization of this invention uses by making the above-mentioned cross linking agent into a gas, as described above. It faces specifically constructing a bridge in the above-mentioned naturally-ocurring-polymers porous body, and fixed time amount bridge formation is performed under the ambient atmosphere of the steam of the cross linking agent saturated with constant temperature in the cross linking agent water solution of fixed concentration. Bridge formation temperature is usually set as 20 degrees C - 50 degrees C that what is necessary is just to select within limits which a living body absorptivity synthetic macromolecule mesh object does not dissolve, and can form the steam of a cross linking agent. Although bridge formation time amount is based also on the class and bridge formation temperature of a cross linking agent, it is desirable to set it as the range in which bridge formation immobilization which does not check the hydrophilic property or living body absorptivity of the above-mentioned naturally-ocurring-polymers porous body, and this thing does not dissolve at the time of a live organ transplant is performed. Bridge formation fixing becomes inadequate, there is a possibility that a naturally-ocurring-polymers porous body may dissolve in a transplantation happiness-in-the-next-life body for a short time, and if bridge formation time amount becomes short, bridge formation-ization will progress so that bridge formation time amount is long, but if bridge formation time amount is too long not much Since the trouble of living body absorptivity also falling is produced, it is not desirable, except that a hydrophilic property becomes low, the seeding consistency to the complex ingredient of the stem cell which specializes in chondrocyte or chondrocyte becomes low and **** and anagenesis of a cell are not performed efficiently.

[0017] When the porous body of living body absorptivity synthetic macromolecule is used in the complex ingredient of this invention, for example, Although this porous body may be beforehand fabricated in the solid configuration corresponding to a transplantation part and the vesicular structure object of naturally-ocurring polymers may be made to form in the hole of this porous body It may be said that such an approach has simple actuation, the stem cell which specializes in chondrocyte or chondrocyte cannot reach the pore of the inner inner part of the vesicular structure object of the above-mentioned naturally-ocurring polymers easily in the case of seeding, and the seeding consistency of these cells becomes low while the mechanical strength is also excellent. The desirable configuration of the composite material of this invention is a sheet-like configuration, and makes the vesicular structure object of naturally-ocurring polymers form in the internal structure matrix of the mesh object of the living body absorptivity synthetic macromolecule of such a configuration, or a porous body, i.e., the friend eye of a mesh object, or the hole of a porous body. The thickness of this whole sheet-like object usually has 0.1-1 preferably desirablemm 0.1-5mm, and although the thickness of the above-mentioned naturally-ocurring-polymers vesicular structure object can be prepared suitably, it is desirable to form in the almost same thickness as the mesh object of living body absorptivity synthetic

macromolecule or a porous body. The voidage of the vesicular structure object is usually 80% or more. In addition, when calling it a sheet-like object in this specification, a film-like thing thru/or a film-like thing are also included.

[0018] For example, in order to manufacture the complex ingredient of the shape of a sheet of this invention as shown in drawing 1 (a), sheet-like the mesh object or porous body of living body absorptivity synthetic macromolecule is located in the center of the water solution of the naturally-ocurring polymers of the living body origin, and is frozen, and it freeze-dries. thereby -- the mesh object or porous body of living body absorptivity synthetic macromolecule -- a naturally-ocurring-polymers vesicular structure -- the complex ingredient of the shape of a sheet sandwiched by the inside of the body is formed. Moreover, sheet-like the mesh object or porous body of living body absorptivity synthetic macromolecule as shown in drawing 1 (b) is frozen on the top face or inferior surface of tongue of a naturally-ocurring-polymers water solution of the living body origin, and if it freeze-dries, the sheet-like complex ingredient whose other sides one side is naturally-ocurring-polymers vesicular structure objects in a living body absorptivity synthetic macromolecule mesh object or a porous body will be formed. In addition, drawing 1 (a) and (b) are mimetic diagrams, according to these, it has indicated that the naturally-ocurring-polymers vesicular structure object is formed in the front face of the mesh object of living body absorptivity synthetic macromolecule, or a porous body, but a naturally-ocurring-polymers vesicular structure object is formed in fact also in the friend eye of the mesh object of living body absorptivity synthetic macromolecule, or the hole of a porous body so that clearly also from the electron microscope photograph of drawing 4 .

[0019] The stem cell which specializes in the chondrocyte and chondrocyte which are used in this invention is prepared from a body tissue with a conventional method. Chondrocyte carries out decomposition processing of the extra-cellular matrix for a living body cartilaginous tissue by enzyme processing of collagenase, a trypsin, RIBARAZE, proteinase, etc., subsequently adds and carries out centrifugal [of the blood serum culture medium], and isolates chondrocyte. The isolated chondrocyte is scattered to a culture flask and it cultivates by the DMEM culture medium (DMEM blood serum culture medium) containing 10% fetal calf serum, a 4500 mg/L glucose, 584mg / L-glutamine, a 0.4mM proline, and 50 mg/L ascorbic acid. Subculture is carried out 2 to 3 times, and trypsinization recovers this cell that carried out subculture, and it considers as the cell sap for seeding until it becomes the sufficient number of cells. Moreover, centrifugal [of the bone marrow extract] is carried out by the density gradient centrifugation using the density gradient medium which consists of PARCOR (percoll), and the stem cell which specializes in chondrocyte isolates it. Subculture is carried out 2 to 3 times until it scatters these cells to a culture flask and becomes the sufficient number of cells by the DMEM blood serum culture medium. Trypsinization recovers the cell which carried out subculture and it considers as the cell sap for seeding.

[0020] For carrying out seeding of the stem cell which specializes in chondrocyte or chondrocyte to the complex ingredient of this invention The above-mentioned complex ingredient is soaked in culture medium, and it carries out by sinking the above-mentioned cell sap for seeding into this complex ingredient, or sinking the cell sap for direct seeding into the above-mentioned complex ingredient. The cell concentration of the above-mentioned cell sap for seeding has 1x106 cells/ml - 5x107 cells/desirable ml, and it is desirable to carry out seeding of the cell sap of the capacity more than the volume of a complex ingredient.

[0021] The transplant for reproducing the cartilaginous tissue of this invention obtains the transplant concerned by adding culture medium, being a DMEM blood serum culture medium and carrying out culture growth of the chondrocyte in this complex in the incubator under 37 degrees C and 5%CO2 ambient atmosphere further, after sinking the above-mentioned cell sap for seeding into the above-mentioned complex ingredient in the case of chondrocyte. After sinking in the cell sap for seeding of the stem cell which the differentiation process to chondrocyte is still more nearly required in the case of a stem cell, and specializes in the above-mentioned complex ingredient at the above-mentioned chondrocyte, The 4500 mg/L glucose after carrying out culture growth for one to two weeks by the DMEM blood serum culture medium, It adds to 584mg / L-glutamine, a 0.4mM proline, and 50 mg/L ascorbic acid, and is a transforming growth

factor. - Cultivate for one to two weeks, it is made to specialize by the DMEM culture medium (differentiation culture medium) containing beta 3 (TGF-beta 3), and the transplant concerned is obtained. Hereafter, when the complex ingredient of this invention is a sheet-like object, seeding of the stem cell which specializes in chondrocyte or chondrocyte is carried out to this sheet-like object, and an example of technique which obtains the transplant for reproducing a cartilaginous tissue is described concretely.

[0022] For example, after paying the sheet-like complex ingredients of this invention into containers, such as a clean and sterile petri dish, and soaking this sheet-like complex ingredient in culture medium, the cell sap for seeding is dropped from a top. Although especially the count that carries out seeding of the cell is not restricted, 1 or 2 times is desirable. When carrying out seeding twice, the 1st time carries out from a top, and the 2nd time is performed after turning a sheet-like complex ingredient over. It is more desirable for the 1st time and the 2nd between to place for 24 hours. In addition, in order to make it the cell by which seeding is carried out not begin to leak from a sheet-like complex ingredient in this case, it is desirable to enclose the edge of a sheet-like complex ingredient in rings, such as rubber. Subsequently, in an incubator, under 37 degrees C and 5%CO2 ambient atmosphere, it puts for 4 hours and cultivates in the condition that the cell sap for seeding sank in into the sheet-like complex ingredient. Then, the ring of rubber is taken out, a lot of culture medium is put in, and after cultivating, the transplant for playback of a cartilaginous tissue is obtained.

[0023] The advantage which uses a sheet-like complex ingredient in this invention originates in the vesicular structure object of naturally-ocurring polymers, such as collagen sponge formed into a complex ingredient, being thin. In a thin vesicular structure object, the above-mentioned cell sap for seeding cannot leak, and can sink into the pore of a porous body, the consistency of the cell held in a complex ingredient as a result will increase, and the rebirth of a cartilaginous tissue will be performed promptly and efficiently. If the complex ingredient which carried out seeding of the stem cell which specializes in chondrocyte or chondrocyte is used with the shape of a sheet, it is possible to reproduce a thin cartilaginous tissue, but as shown in drawing 2 , the laminating of the complex ingredient which carried out seeding of these cells can be carried out, and it can also be used. In this case, the number of sheets in which a sheet-like complex ingredient carries out a laminating can adjust the thickness of the cartilage reproduced. Since seeding of the above-mentioned cell is performed in each of the sheet-like complex ingredient with which the laminating of the thing of this drawing 2 was carried out, if it is high and transplants by making this into a transplant in the living body, without the consistency of the cell by which seeding was carried out being different from the sheet-like complex ingredient of one sheet, the rebirth of a cartilaginous tissue will be performed good.

[0024] Moreover, as shown in drawing 3 , the sheet-like complex ingredient which carried out seeding of the cell can be rolled in the shape of a roll, and it can also be made a cylinder-like configuration. In this case, the die length of the cartilage reproduced is the height of a roll, and a diameter can be adjusted by the count which rolls a roll. Furthermore, in this invention, it is also possible to double with the configuration of the deficit section of a cartilaginous tissue, and to deform or gather the above-mentioned sheet-like object suitably. It is more desirable to cultivate from five days before these shaping to two weeks, in order to obtain the transplant of various configurations, such as laminated material of said sheet-like composite material or a roll-like object.

[Example] Hereafter, an example explains this invention to a detail further.

[0025] Example 1 mechanical strength was immersed in 0.5wt(s)% cow I-beam atelocollagen aqueous acids (pH=3.0), and froze the copolymer (PLGA) mesh object of the lactic acid and glycolic acid which are a high bioabsorbable polymer at -80 degrees C for 12 hours. Next, this freezing object was freeze-dried under vacuum reduced pressure (0.2 Torr) for 24 hours, and the non-bridge complex ingredient of the shape of a sheet of a PLGA mesh object and collagen sponge was manufactured. After carrying out bridge formation processing of the obtained non-constructed bridge compound biomaterial for 4 hours with the glutaraldehyde steam saturated with 37 degrees C in the 25wt% glutaraldehyde water solution, the phosphate buffer solution washed 10 times. Furthermore, after it was immersed in the 0.1M glycine water solution for 4

hours and the phosphate buffer solution washed 10 times, distilled water washed 3 times and it froze at -80 degrees C for 12 hours. This was freeze-dried under vacuum reduced pressure (0.2 Torr) for 24 hours, and the bridge complex of the shape of a sheet of the PLGA mesh object of this invention and collagen sponge was obtained. This ingredient was coated with gold and those structures were observed with the scanning electron microscope (SEM). The result is shown in drawing 4 .

[0026] The bridge complex ingredient of the shape of a sheet of the PLGA mesh object and collagen sponge which were obtained in the example 2 example 1 was cut in the magnitude whose width of face is 5.0mm and whose die length is 20.0mm, and the sample was produced. The static tension test was performed in the condition of it having been immersed in 2-[4-(2-hydroxyethyl)-1-PIPERAJIRU] ethane sulfone (HEPES) buffer solution (pH7.4), and having got this sample wet. The result is shown in Table 1.

[0027]

[Table 1]

試験サンプル	静的ヤング率 (Kpa)
PLGA-コラーゲン	35.42±1.43
PLGA*	35.15±1.00
コラーゲンスポンジ**	0.02±0.00

* comparison sample: -- PLGA mesh object independent ** comparison sample: used in the example 1 -- 0.5wt% cow I-beam atelocollagen aqueous acids (pH=3.0)

The collagen independent sponge table 1 obtained in independent by [the / as an example 1 / same] carrying out porous bridge formation processing shows that higher tensile strength is shown compared with the biomaterial which consists only of collagen sponge after the bridge complex ingredient of the PLGA mesh object of this invention and collagen sponge has been damp with the HEPES buffer solution, and the almost same tensile strength as a PLGA mesh is shown in it.

[0028]

[Example 3] Sheet-like bridge complex of the PLGA mesh object and collagen sponge which were created in the example 1 It sterilized by ethylene oxide gas. After, shaving and taking down the thin piece of a cartilage from the cartilage of a cow elbow joint to Metz on the other hand and cutting fine finely, it incubated at 37 degrees C in the DMEM culture medium containing the collagenase of 0.2(w/v) % for 12 hours. And the at-long-intervals alignment of the supernatant filtered with the nylon filter whose pore size is 70 micrometers was carried out by 2000rpm for 5 minutes, and after washing twice by the antibiotic and the DMEM blood serum culture medium which contains fetal calf serum 10%, the chondrocyte of a cow elbow was obtained. The obtained chondrocyte was cultivated under 37 degrees C and 5%CO2 ambient atmosphere by the DMEM blood serum culture medium. The chondrocyte which carried out subculture twice was exfoliated and collected by EDTA/PBS (-) 0.025% trypsin / 0.01%, and 1x107 cells/ml cell sap was prepared. Next, the sheet-like bridge complex of the above-mentioned PLGA mesh object and collagen sponge which sterilized by ethylene oxide gas was soaked in the DMEM blood serum culture medium, the edge of complex (film) was enclosed in the ring of rubber, and 1.3ml[/cm] 2 cell sap was dropped. In the incubator, under 37 degrees C and 5%CO2 ambient atmosphere, it put for 4 hours and cultivated. Then, the ring of rubber was removed, a lot of culture medium was put in, and culture was continued. Culture media were exchanged for every three days. Complex was transplanted to hypodermically [of the back of a nude mouse] after cultivating for one week. Recovery of the specimen was carried out after transplantation at the time for six weeks, and HE (hemato oxy-phosphorus and eosine) dyeing and safranin-O dyeing were performed. Moreover, type which collects m-RNA and is seen by RT-PCR peculiar to an articular cartilage organization from a specimen II A collagen and manifestation analysis of AGURIKAN were performed.

[0029] Consequently, the specimen transplanted to hypodermically [of the back of a nude mouse] has after [six weeks] surface gloss, as shown in drawing 5 , and it was observed that a

color is opalescence. Moreover, as a result of performing hematoxylin eosin staining and safranin-O dyeing, as a specimen was shown in drawing 6 , the small-circle form cell in a lacuna and the Safranin-O dye affinity extra-cellular matrix were accepted. It types in the m-RNA sample furthermore extracted from the specimen. II Detection identification of m-RNA which discovers a collagen and AGURIKAN was carried out, and the reproduced organization checked that he was an articular cartilage organization by these things.

[0030]

[Effect of the Invention] In this invention, the complex ingredient used as support support of the stem cell which specializes in chondrocyte or chondrocyte has the structure where the living body absorptivity synthetic macromolecule mesh object and the naturally-ocurring-polymers porous body of the living body origin were firmly formed into bridge formation compound. A living body absorptivity synthetic macromolecule mesh object functions as a mechanical frame, and an advantageous configuration controllability, the outstanding mechanical strength, and the ease of handling are granted, and the naturally-ocurring-polymers porous body is extremely excellent in the differentiation and growth of a stem cell which specialize in growth or the cartilage of chondrocyte, and the above-mentioned complex ingredient of this invention has these outstanding properties. Moreover, the complex ingredient fabricated especially in the shape of a sheet has good seeding effectiveness, and the consistency of the above-mentioned cell you are made to support in a complex ingredient is very high, and when this complex ingredient is made by this to support the stem cell which specializes in growth of chondrocyte, or chondrocyte and it transplants to a living body, the rebirth of a cartilaginous tissue is prompt and very efficient. Therefore, the transplant which made the complex ingredient of this invention and this support the stem cell which specializes in chondrocyte or chondrocyte is very useful as a playback means of a cartilaginous tissue.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] (a) It is the type section Fig. of the typical sheet-like complex ingredient which reaches and (b) requires for this invention.
[Drawing 2] **, the lengthwise direction type section Fig. of the sheet-like complex ingredient by which the laminating was carried out.
[Drawing 3] **, the longitudinal direction type section Fig. of the sheet-like complex ingredient rolled in the shape of a roll.
[Drawing 4] The electron microscope photograph of ** and the ** sheet-like complex ingredient of this invention.
[Drawing 5] **, the reproduced appearance photograph of a cow articular cartilage organization.
[Drawing 6] **, the reproduced histological dyeing photograph of a cow articular cartilage organization.

[Translation done.]

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最終頁に続く

(54)【発明の名称】　軟骨組織の再生用移植体

(57)【要約】
【課題】優れた生体親和性を示し、細胞の播種性、臨床での取り扱い性に優れ、しかも十分な機械強度を有する、軟骨組織を再生するための移植体を提供する。
【解決手段】生体吸収性合成高分子からなるメッシュ体あるいは多孔質体の内部構造マトリックス内に天然高分子多孔質構造体を形成して細胞担持用担体を得、当該担体に軟骨細胞あるいは軟骨細胞に分化する幹細胞を担持して移植体を構成する。

【特許請求の範囲】
【請求項1】生体吸収性合成高分子からなるメッシュ体あるいは多孔質体の内部構造マトリックス内に、さらに天然高分子からなる多孔質構造体が形成されている複合体材料からなる、軟骨細胞あるいは軟骨細胞に分化する幹細胞の担持用担体。
【請求項２】複合体材料がシート状物である、請求項１に記載の軟骨細胞あるいは軟骨組織に分化する幹細胞の担持用担体。
【請求項３】複合体材料が請求項２に記載のシート状物を積層またはロール状に巻いたものである、請求項１の軟骨細胞あるいは軟骨組織に分化する幹細胞の担持用担体。
【請求項４】請求項１～３のいずれか１項記載の軟骨細胞あるいは軟骨細胞に分化する幹細胞の担持用担体に、軟骨細胞あるいは軟骨細胞に分化する幹細胞を担持せしめた、軟骨組織再生に用いるための移植体。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】本発明は、変形性関節症などの疾患や事故などの原因による軟骨創傷を修復するために用いられる軟骨組織の再生に関する。
【０００２】
【従来の技術】変形性関節症は整形外科分野において高頻度見られる疾患で、しばしば高度の機能障害をきたす。人工関節手術が外科的治療の主体を成すが、現在の金属や高分子ポリマーを材料とする人工関節部分は感染・磨耗・緩み・破損といった問題を有する。組織移植の場合では、ドナーの不足という問題に加え、ドナーが他人の場合、免疫応答に基づく拒絶反応という問題もある。このような種々の問題点の存在により、現在では、再生医工学的な手法による治療法は理想的であると考えれ、軟骨組織の再生に関する研究が盛んに行われている。再生医工学的な手法により軟骨組織を再生するためには、軟骨細胞あるいは軟骨細胞に分化する幹細胞が増殖するための足場として、また、形成している生体組織の支持体としての3次元的な多孔質性の担体材料が必要である。このような担体材料は多孔質性や生体親和性や生体吸収性などの条件が要求され、従来、ポリ乳酸（PLA）やポリグリコール酸（PGA）や乳酸とグリコール酸との共重合体(PLGA)のような生体吸収性合成高分子、あるいはコラーゲンなどの天然高分子で調製した3次元的な多孔質性担体材料がよく用いられている。
【０００３】しかしながら、上記の生体吸収性合成高分子からなるものは、機械強度に優れているものの、疎水性であり、またその大きい隙間、割れ目のため、大部分の細胞は隙間を通過してしまい、その上に載らず、軟骨細胞あるいは軟骨細胞に分化する幹細胞を播種することが極めて困難であり、このため、有効な細胞の播種率が得られず、これら細胞を大量に支持担体に集積することができないため、軟骨組織の再生効率が低く、実用上大きな障害となっていた。一方、生体由来の天然高分子の多孔質性材料である例えばコラーゲンスポンジは、親水性で、細胞との相互作用が非常に優れており、細胞の播種の容易なものであるが、機械強度が低く、また柔らかくて捩れやすいので、臨床では取り扱いにくいという問題点を包含していた。
【０００４】
【発明が解決しようとする課題】本発明は、従来技術のこのような問題点を解決することを課題とするものである。　　具体的には、良好な生体親和性を有し、軟骨細胞あるいは軟骨細胞に分化する幹細胞の播種が容易で、播種効率が良好であり、そのためこれら細胞を大量に支持担体に集積することが可能であって、軟骨組織の再生効率が良好であるとともに、機械的強度も高く、臨床においても取扱い易い、軟骨細胞あるいは軟骨細胞に分化する幹細胞の支持担体を提供することにあり、さらに、このような材料に軟骨細胞あるいは軟骨細胞に分化する幹細胞を含有せしめた、軟骨組織を再生するための生体内移植体を提供しようとするものである。
【０００５】
【問題を解決するための手段】本発明は、上記の課題を解決するためになされたものであり、以下（１）～（４）からなるものである。
（1）生体吸収性合成高分子からなるメッシュ体あるいは多孔質体の表面および内部構造マトリックス内に、さらに天然高分子からなる多孔質構造体が形成されている複合体材料からなる、軟骨細胞あるいは軟骨細胞に分化する幹細胞の担持用担体。
（２）複合体材料がシート状物である、（１）に記載の軟骨細胞あるいは軟骨組織に分化する幹細胞の担持用担体。
（３）複合体材料が上記（２）に記載のシート状物を積層またはロール状に巻いたものである、（１）の軟骨細胞あるいは軟骨細胞に分化する幹細胞の担持用担体。
（４）（１）～（３）いずれか１に記載の軟骨細胞あるいは軟骨細胞に分化する幹細胞の担持用担体に、軟骨細胞あるいは軟骨細胞に分化する幹細胞を担持せしめた、軟骨組織再生に用いるための移植体。
【０００６】以下、本発明をさらに詳述する。本発明における軟骨細胞あるいは軟骨細胞に分化する幹細胞を担持する担体は、生体吸収性合成高分子のメッシュ体あるいは多孔質体における表面およびその内部構造マトリックス内にコラーゲン等の天然高分子からなる多孔質構造体をさらに形成した複合体材料により構成される。
【０００７】本発明に用いられる生体吸収性合成高分子のメッシュ体あるいは多孔質体は、主として本発明の複合体材料の機械的強度を増大させるために用いられ、メッシュ体は、織物、織布又は不織布等からなるものでよい。また、多孔質体は、発泡剤を利用する発泡成形法、

あるいは多孔質化剤除去法等周知の方法により得ることができる。この多孔質体の発泡成型法においては、高分子化合物に発泡剤を添加し発泡剤を発泡させた後、上記高分子を硬化させる。高分子溶液中に、水溶性の糖類あるいは塩類を添加し、硬化後、該水溶性物質を水で洗浄除去すればよい。メッシュのアミ目の大きさあるいは多孔質体の孔の大きさは大きくなればなるほど、機械的強度は低下するものの、メッシュ単位当たりの天然高分子多孔質構造体の細孔密度が高くなり、播種細胞はこの細孔に保持されるので、複合体における播種細胞数を増大でき、軟骨組織の再生が効率的になる。したがって、そのメッシュのアミ目の大きさあるいは多孔質体の孔の大きさは、移植される生体内の場所等に応じて、求められる機械強度あるいは弾力性、あるいは軟骨組織の再生速度等を勘案して適宜定められる。

【０００８】メッシュ体あるいは多孔質体を形成する生体吸収性合成高分子としては、ポリ乳酸、ポリグリコール酸、乳酸とグリコール酸の共重合体、ポリリンゴ酸、ポリーεーカプロラクトンなどのポリエステル或いはセルロース、ポリアルギン酸などの多糖類等を挙げることができる。本発明において好ましく使用される生体吸収性合成高分子はポリ乳酸、ポリグリコール酸、乳酸とグリコール酸の共重合体である

【０００９】本発明の天然高分子多孔質構造体は、生体に由来するもので、生体親和性を示すものであれば、何れも使用できるが、コラーゲン、ゼラチン、フィブロネクチン、及びラミニンから選ばれた１種以上のもの、特にコラーゲンが好ましく使用される。コラーゲンにはI、II、III、IV型のものがあるが、本発明においてはこれらの何れも使用できる。天然高分子多孔質構造体の細孔は、播種細胞の増殖及び組織再生の足場とするものであり、細孔は連続していることが好ましい。その大きさは1～300μm、好ましくは20～100μm程度とするのがよい。また、本発明においては、厚みは、生体複合材料の使用態様によって適宜定めればよいが、通常0.１～5mm、好ましくは0.１～１mmである。その空隙率は、通常８０％以上である。

【００１０】本発明の複合体材料は、生体吸収性合成高分子のメッシュ体あるいは多孔質体の内部構造マトリックス内、すなわちメッシュ体のアミ目あるいは多孔質体の孔内に、コラーゲン等の天然高分子からなる多孔質体をさらに形成したものであり、本発明の複合体材料は種々の方法により製造することができるが、例えば、前記生体吸収性合成高分子のメッシュ体あるいは多孔質体と天然高分子多孔質構造体とを架橋結合させることにより得ることができる。この方法は、（１）生体吸収性合成高分子のメッシュ体あるいは多孔質体にコラーゲン等の天然高分子材料の溶液を付着、含浸せしめた後、（２）凍結乾燥し、次いで、（３）生成する生体複合材料をガス状の化学架橋剤で処理するものである。上記工程（１）においては、前記生体吸収性合成高分子メッシュ体を前記生体由来の天然高分子水溶液で処理する。処理方法としては種々のものがあるが、浸漬法や塗布法が好ましく採用される。浸漬法は、生体由来の天然高分子水溶液の濃度や粘度が低い場合に有効であり、具体的には、生体由来の天然高分子の低濃度水溶液に生体吸収性合成高分子メッシュ体を浸漬することにより行われる。塗布法は、生体由来の天然高分子水溶液の濃度や粘度が高く、浸漬法が適用できないときに有効であり、具体的には、生体由来の天然高分子の高濃度水溶液を生体吸収性合成高分子メッシュ体に塗布することにより行われる。

【００１１】生体吸収性合成高分子メッシュ体あるいは多孔質体に天然高分子溶液が含浸、付着した複合物は、次いで（２）凍結乾燥に付される。凍結乾燥は、上記複合物を凍結し、これを真空減圧下で凍結乾燥するものであるが、かかる工程により、生体由来の天然高分子が多孔質化され、生体吸収性合成高分子のメッシュ体あるいは多孔質体と天然高分子多孔質構造体との複合体材料が形成される。

【００１２】凍結乾燥の方法は、従来公知の方法がそのまま適用できる。凍結温度は、通常－２０℃以下である。凍結乾燥圧力は、凍結された水が気体となる減圧条件を設定すればよく、通常、０．２Ｔｏｒｒ程度の減圧下に調製される。凍結乾燥された複合体材料は、ついで（３）の架橋工程に付される。この工程は、複合生体材料を構成する生体由来の天然高分子多孔体をガス状の架橋剤により、架橋化し、天然高分子の多孔質構造体を固めると共に合成高分子メッシュ体との結合力を高め、所望とする架橋複合体材料の多孔質構造を安定するための十分な弾力と強度を与えるために必要なものである。

【００１３】一般に、架橋化方法としては、紫外線照射処理による光架橋や熱架橋などの物理的架橋法、溶液状の架橋化剤やガス状の架橋化剤を用いる化学架橋法などが知られているが、本発明においては、ガス状の架橋化剤を用いる方法が最も望ましい。

【００１４】すなわち、紫外線照射処理による光架橋や熱架橋などの物理的架橋法では、その架橋化工程において、架橋度は限られて、更には複合生体材料を構成する生体吸収性合成高分子の変質や分解を惹起する恐れがあり、また化学架橋法でも、溶液状の架橋化剤を用いる方法では、その架橋過程で天然高分子が溶解する恐れがあるからである。なお、天然高分子の架橋化剤溶液の溶解を防止するめに、溶液状の架橋化剤による架橋に先だって光架橋や熱架橋を施す方法を採用したとしても、前記したように光や熱により天然高分子の分解や変質が生じてしまうので望ましくない。

【００１５】これに対して、ガス状の架橋剤を用いる方法は、上記のような問題点の全てが克服され、分解や変質を生じることなく、天然高分子が所望の形態で架橋さ

れ、３次元化されると共に合成高分子メッシュ体との結合力を高められ、目的とするに足る十分な強度と弾力を有する架橋化された複合体材料を得ることができる。本発明で用いられる架橋剤としては、従来公知のものが何れも使用できる。好ましく使用される架橋剤は、グルタルアルデヒド、ホルムアルデヒド、パラホルムアルデヒドのようなアルデヒド類、特にグルタルアルデヒドである。

【００１６】本発明の架橋化は、前記したように、上記の架橋剤をガス状にして用いる。具体的には、上記天然高分子多孔質体を架橋するに際し、一定温度で一定濃度の架橋剤水溶液で飽和した架橋剤の蒸気の雰囲気下で一定時間架橋を行う。架橋温度は、生体吸収性合成高分子メッシュ体が溶解せず、且つ架橋剤の蒸気が形成できる範囲内で選定すればよく、通常、２０℃～５０℃に設定される。架橋時間は、架橋剤の種類や架橋温度にもよるが、上記天然高分子多孔質体の親水性や生体吸収性を阻害せず、かつ生体移植時にこのものが溶解しないような架橋固定化が行われる範囲に設定するのが望ましい。架橋時間が短くなると、架橋固定化が不十分となり、移植後生体内で天然高分子多孔質体が短時間で溶解する恐れがあり、また架橋時間が長いほど架橋化が進むが、架橋時間があまり長過ぎると、親水性が低くなり、軟骨細胞あるいは軟骨細胞に分化する幹細胞の複合体材料に対する播種密度が低くなり、細胞の贈殖及び組織再生が効率よく行われないほか、生体吸収性も低下する等の問題点を生じるので好ましくない。

【００１７】本発明の複合体材料においては、例えば生体吸収性合成高分子の多孔質体を用いる場合、移植部位に対応する立体形状にあらかじめ該多孔質体を成形し、この多孔質体の孔に天然高分子の多孔質構造体を形成せしめてもよいが、このような方法は操作が簡便であり、機械的強度も優れている反面、軟骨細胞あるいは軟骨細胞に分化する幹細胞が、播種の際、上記天然高分子の多孔質構造体の内奥部の細孔に到達しにくく、これら細胞の播種密度が低くなるということがある。本発明の複合材料の望ましい形状は、シート状の形状であり、このような形状の生体吸収性合成高分子のメッシュ体あるいは多孔質体の内部構造マトリックス内、すなわちメッシュ体のアミ目あるいは多孔質体の孔内に、天然高分子の多孔質構造体を形成させる。このシート状物の全体の厚さは、通常０．１～５mm、好ましくは０．１～１mmが好ましく、また、上記天然高分子多孔質構造体の厚さは適宜調製できるが、生体吸収性合成高分子のメッシュ体あるいは多孔質体とほぼ同じ厚さに形成するのが好ましい。その多孔質構造体の空隙率は、通常８０％以上である。なお、本明細書においてシート状物というときは、フィルム状のものないし膜状のものも包含する。

【００１８】例えば、図１（ａ）に示されるような、本発明のシート状の複合体材料を製造するには、シート状の生体吸収性合成高分子のメッシュ体あるいは多孔質体を生体由来の天然高分子の水溶液の中央に位置させて凍結し、凍結乾燥する。これにより、生体吸収性合成高分子のメッシュ体あるいは多孔質体が天然高分子多孔質構造体中にサンドイッチされているシート状の複合体材料が形成される。　また、図１（ｂ）に示されるような、シート状の生体吸収性合成高分子のメッシュ体あるいは多孔質体を生体由来の天然高分子水溶液の上面或いは下面で凍結し、凍結乾燥すると、片面が生体吸収性合成高分子メッシュ体あるいは多孔質体で、他面が天然高分子多孔質構造体であるシート状複合体材料が形成される。なお、図１（ａ）および（ｂ）は、模式図であり、これらによれば、天然高分子多孔質構造体が生体吸収性合成高分子のメッシュ体あるいは多孔質体の表面に形成されているように記載しているが、図４の電子顕微鏡写真からも明らかなように、実際には、天然高分子多孔質構造体は、生体吸収性合成高分子のメッシュ体のアミ目あるいは多孔質体の孔内にも形成される。

【００１９】本発明において用いる軟骨細胞および軟骨細胞に分化する幹細胞は常法により生体組織から調製する。軟骨細胞は、生体軟骨組織をコラーゲナーゼ、トリプシン、リバラーゼ、プロティナーゼ等の酵素処理により、細胞外マトリックスを分解処理し、次いで血清培地を添加し、遠心して、軟骨細胞を単離する。単離した軟骨細胞を培養フラスコに播き、１０％ウシ胎児血清，４５００mg／Lグルコース、５８４mg／Lグルタミン、０．４mMプロリンおよび５０mg／Lアスコルビン酸を含有するＤＭＥＭ培地（ＤＭＥＭ血清培地）で培養する。十分な細胞数になるまで、２～３回継代培養し、この継代培養した細胞をトリプシン処理により回収し、播種用細胞液とする。また、軟骨細胞に分化する幹細胞は、骨髄抽出液をパーコール（ｐｅｒｃｏｌｌ）からなる密度勾配媒体を用いた密度勾配遠心法により遠心して単離する。これらの細胞を培養フラスコに播き、ＤＭＥＭ血清培地で十分な細胞数となるまで、２～３回継代培養する。継代培養した細胞をトリプシン処理により回収し、播種用細胞液とする。

【００２０】本発明の複合体材料に、軟骨細胞あるいは軟骨細胞に分化する幹細胞を播種するには、　上記複合体材料を培養液で濡らしておき、この複合体材料に上記播種用細胞液を含浸するか、あるいは上記複合体材料に直接播種用細胞液を含浸することにより行う。上記播種用細胞液の細胞濃度は、1×10^6cells／ｍｌ～5×10^7cells／ｍｌが望ましく、複合体材料の体積以上の容量の細胞液を播種することが望ましい。

【００２１】本発明の軟骨組織を再生するための移植体は、軟骨細胞の場合、上記複合体材料に上記播種用細胞液を含浸した後、さらに、培養液を添加し、該複合体中の軟骨細胞をＤＭＥＭ血清培地で、３７℃、５％CO_2雰囲気下のインキュベータにおいて培養増殖させること

により、当該移植体を得る。幹細胞の場合は、さらに軟骨細胞への分化工程が必要であり、上記複合体材料に上記軟骨細胞に分化する幹細胞の播種用細胞液を含浸した後、ＤＭＥＭ血清培地で１～２週間培養増殖させた後、４５００ｍｇ／Ｌグルコース、５８４ｍｇ／Ｌグルタミン、０．４ｍＭプロリンおよび５０ｍｇ／Ｌアスコルビン酸に加え、トランスフォーミング増殖因子－β３（ＴＧＦ－β３）を含有するＤＭＥＭ培地（分化培地）で１～２週間培養し、分化させて当該移植体を得る。以下、本発明の複合体材料がシート状物である場合において、軟骨細胞あるいは軟骨細胞に分化する幹細胞を該シート状物に播種し、軟骨組織を再生するための移植体を得る手法の一例について具体的に述べる。

【００２２】例えば、本発明のシート状複合体材料を清潔で無菌なシャーレ等の容器中に入れ、該シート状複合体材料を培養液で濡らしてから、播種用細胞液を上から滴下する。細胞を播種する回数は特に限らないが、1、２回が望ましい。２回播種する時、１回目が上から行って、２回目はシート状複合体材料を裏返してから行う。１回目と２回目の間が２４時間置いたほうが望ましい。なお、この際、播種される細胞が、シート状複合体材料から漏れ出さないようにするため、シート状複合体材料の縁をゴム等のリングで囲っておくことが望ましい。次いでシート状複合体材料中に播種用細胞液が含浸された状態で、インキュベータ中において３７℃、５％ＣＯ２雰囲気下で4時間静置して培養する。その後、ゴムのリングを取り出し、多量の培養液を入れて、培養した後、軟骨組織の再生用移植体を得る。

【００２３】本発明においてシート状の複合体材料を使用する利点は、複合体材料中に形成されるコラーゲンスポンジ等の天然高分子の多孔質構造体が薄いことに起因する。薄い多孔質構造体においては、上記播種用細胞液が多孔質体の細孔にもれなく含浸でき、結果として複合体材料において保持される細胞の密度が高まり、軟骨組織の再生が速やかにかつ効率的に行われることになる。軟骨細胞あるいは軟骨細胞に分化する幹細胞を播種した複合体材料をシート状のままで用いると、薄い軟骨組織を再生することが可能であるが、図２に示すように、これら細胞を播種した複合体材料を積層して用いることもできる。この場合において再生される軟骨の厚みはシート状複合体材料の積層する枚数により調整できる。この図２のものは、積層されたシート状複合体材料の各々において上記細胞の播種が行われているので、播種された細胞の密度は、１枚のシート状複合体材料と変わらずに高く、これを移植体として生体内に移植すれば、軟骨組織の再生が良好に行われる。

【００２４】また、図３に示すように、細胞を播種したシート状複合体材料をロール状に巻いて、円筒状の形状にすることもできる。この場合においては、再生される軟骨の長さは、ロールの高さで、直径はロールを巻く回数で調整できる。さらに、本発明においては、軟骨組織の欠損部の形状に合わせて、上記シート状物を適宜変形あるいは集合させることも可能である。前記シート状複合材料の積層物あるいはロール状物等の種々の形状の移植体を得るには、これらの成形前の５日間から２週間まで培養したほうが望ましい。

【実施例】以下、本発明を実施例により更に詳細に説明する。

【００２５】実施例１

機械強度が高い生体吸収性高分子である乳酸とグリコール酸との共重合体(PLGA)メッシュ体を0.5wt％のウシI型アテロコラーゲン酸性水溶液（pH＝3.0）に浸漬し、-80℃で12時間凍結した。次にこの凍結物を、真空減圧下（0.2 Torr）で24時間凍結乾燥し、PLGAメッシュ体とコラーゲンスポンジとのシート状の未架橋複合体材料を製造した。得られた未架橋複合生体材料を37℃で、25wt％のグルタルアルデヒド水溶液で飽和したグルタルアルデヒド蒸気で4時間架橋処理した後、リン酸緩衝液で10回洗浄した。さらに、0.1Mグリシン水溶液に4時間浸漬し、リン酸緩衝液で10回洗浄した後、蒸留水で3回洗浄し、-80℃で12時間凍結した。これを真空減圧下（0.2 Torr）で24時間凍結乾燥し、本発明のPLGAメッシュ体とコラーゲンスポンジとのシート状の架橋複合体を得た。この材料を金でコーティングし、それらの構造を走査型電子顕微鏡（SEM）で観察した。その結果を図４に示す。

【００２６】実施例２

実施例１で得た、PLGAメッシュ体とコラーゲンスポンジとのシート状の架橋複合体材料を幅が５．０mm、長さが２０．０mmの大きさに切り、サンプルを作製した。このサンプルを２－［４－（２－ヒドロキシエチル）－１－ピペラジル］エタンスルホン（HEPES）緩衝液（pH 7.4）に浸漬し濡れた状態で、静的引張試験を行った。その結果を表１に示す。

【００２７】

【表１】

試験サンプル	静的ヤング率（Kpa）
PLGA-コラーゲン	35.42±1.43
PLGA*	35.15±1.00
コラーゲンスポンジ**	0.02±0.00

＊比較サンプル：実施例1で用いたPLGAメッシュ体単独

＊＊比較サンプル：0.5wt％のウシI型アテロコラーゲン酸性水溶液（pH＝3.0）単独を実施例1と同様の多孔架橋処理することにより得

られたコラーゲン単独スポンジ表１から、本発明のＰＬＧＡメッシュ体とコラーゲンスポンジとの架橋複合体材料は、HEPES緩衝液で濡れた状態で、コラーゲンスポンジのみからなる生体材料に比べより高い引張強度を示し、また、PLGAメッシュとほぼ同じな引張強度を示すことが判る。

【００２８】

【実施例３】実施例１で作成したPLGAメッシュ体とコラーゲンスポンジとのシート状架橋複合体を　酸化エチレンガスで滅菌した。一方、ウシ肘関節の軟骨から薄い軟骨片をメスで剃りおろし、細かく刻んだ後、0.2（w/v）％のコラーゲナーゼを含有するDMEM培地中で３７℃で１２時間インキュベートした。そして、ポアサイズが７０μmのナイロンフィルターで濾過した上澄みを2000rpmで５分間遠心し、抗生物質と１０％ウシ胎児血清を含有するDMEM血清培地で２回洗浄した後、ウシ肘の軟骨細胞を得た。得られた軟骨細胞をDMEM血清培地で３７℃、５％CO_2雰囲気下で培養した。２回継代培養した軟骨細胞を0.025％トリプシン/0.01％EDTA/PBS（-）で剥離・採集し、1×10^7cells/ml細胞液を調製した。次に、酸化エチレンガスで滅菌した上記PLGAメッシュ体とコラーゲンスポンジとのシート状架橋複合体をDMEM血清培地で濡らし、複合体（膜）の縁をゴムのリングで囲って、１．３ml/cm^2細胞液を滴加した。インキュベータ中で、３７℃、５％CO_2雰囲気下で、4時間静置して培養した。その後、ゴムのリングを取り外し、多量の培養液を入れて、培養を続けた。培地を三日間ごとに交換した。1週間培養した後、複合体をヌードマウスの背中の皮下に移植した。移植後6週の時点で検体を採収し、ＨＥ（ヘマトオキシリンとエオシン）染色、safranin-O染色を行った。また、検体よりｍ－ＲＮＡを回収しRT-PCRにより関節軟骨組織に特有にみられるタイプ II コラーゲン、アグリカンの発現解析を行った。

【００２９】その結果、ヌードマウスの背中の皮下に移植した検体は、図５に示すように6週間後表面光沢があり、色が乳白色であることが観察された。また、検体をHE染色とsafranin-O染色を行った結果、図６に示すように、小窩内小円形細胞とSafranin-O染色性細胞外マトリックスが認められた。さらに検体から抽出したｍ－ＲＮＡ試料中にタイプ II コラーゲンやアグリカンを発現するｍ－ＲＮＡが検出同定され、これらのことにより、再生した組織が関節軟骨組織であることを確認した。

【００３０】

【発明の効果】本発明において、軟骨細胞あるいは軟骨細胞に分化する幹細胞の担持担体として使用する複合体材料は、生体吸収性合成高分子メッシュ体と生体由来の天然高分子多孔質体が強固に架橋複合化された構造を有するものである。生体吸収性合成高分子メッシュ体は、メカニカル骨格として機能し、有利な形状制御性、優れた機械的強度及び取扱いの容易性を賦与し、また、天然高分子多孔質体は、軟骨細胞の増殖あるいは軟骨に分化する幹細胞の分化および増殖に極めて優れており、本発明の上記複合体材料は、これらの優れた特性を併せ持つものである。また、特にシート状に成形された複合体材料は、播種効率が良好で、複合体材料中に担持せしめられる上記細胞の密度が極めて高く、これにより、この複合体材料に軟骨細胞の増殖あるいは軟骨細胞に分化する幹細胞を担持せしめて、生体に移植した場合、軟骨組織の再生は速やかで、極めて効率的である。したがって、本発明の複合体材料およびこれに軟骨細胞あるいは軟骨細胞に分化する幹細胞を担持せしめた移植体は、軟骨組織の再生手段として極めて有用なものである。

【図面の簡単な説明】

【図１】（ａ）および（ｂ）は、本発明に係る代表的なシート状複合体材料の模式断面図。

【図２】は、積層されたシート状複合体材料の縦方向模式断面図。

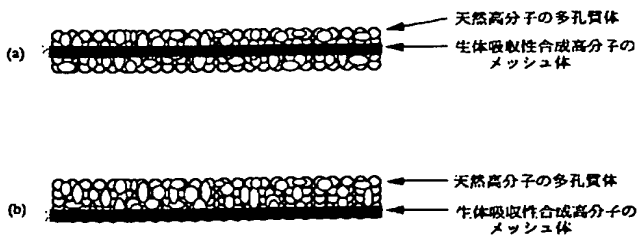
【図３】は、ロール状に巻かれたシート状複合体材料の横方向模式断面図。

【図４】は、本発明のるシート状複合体材料の電子顕微鏡写真。

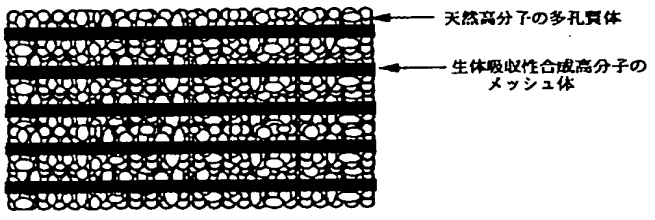
【図５】は、再生したウシ関節軟骨組織の外観写真。

【図６】は、再生したウシ関節軟骨組織の組織学的な染色写真。

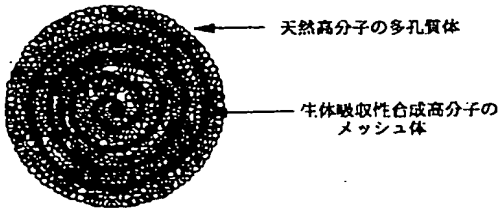
【図１】



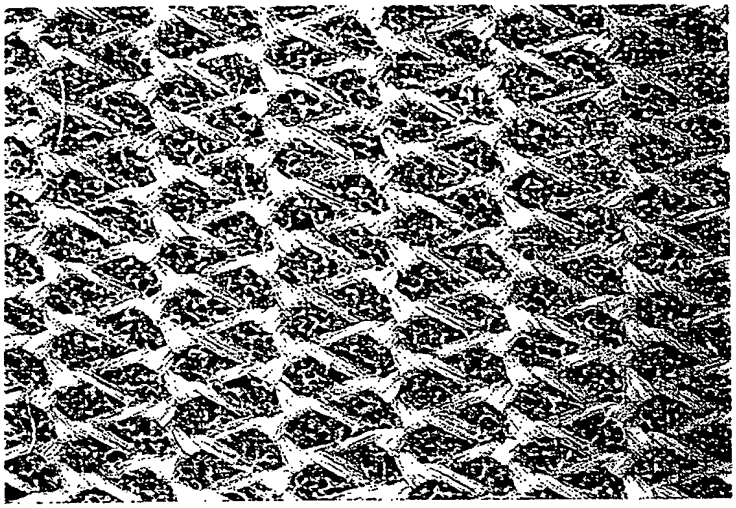
【図２】



【図３】



【図４】



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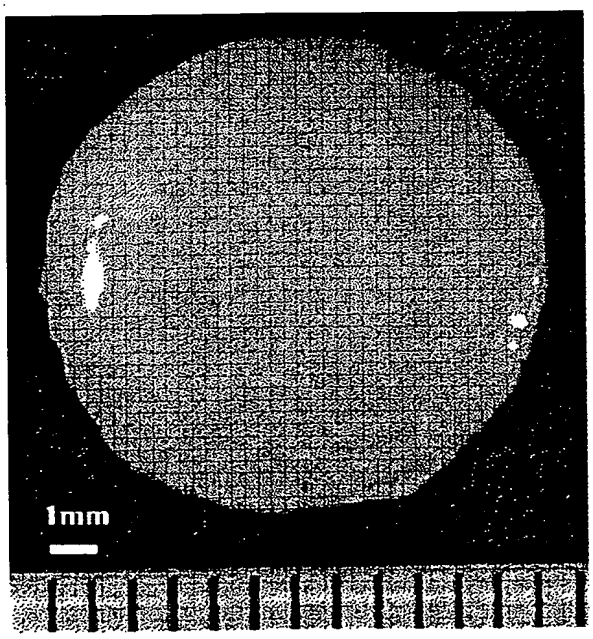
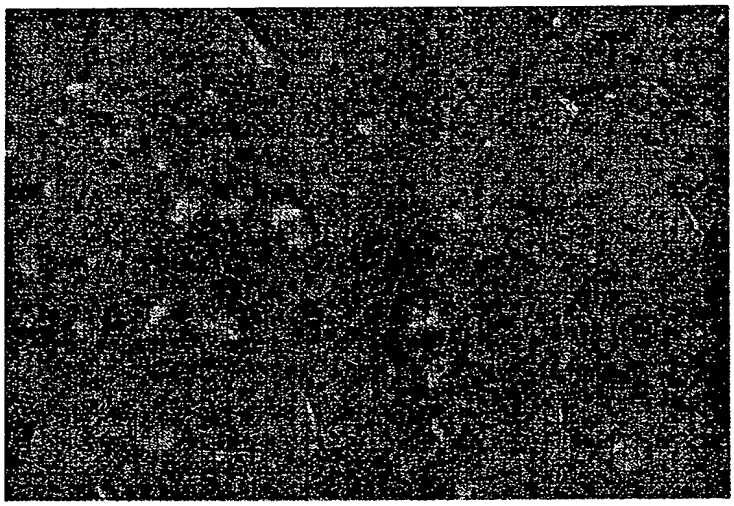


【図６】

safranin-O染色



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Ｆターム(参考) 4B065 AA90X BA22 BD39 BD40
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